JOURNAL OF THE NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

EDITED, PRINTED AND PUBLISHED FOR THE ASSOCIATION

ΒY

DOUGLAS WHILLANS

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JOURNAL

of the

NEW ZEALAND ASSOCIATION OF BACTERIOLOGISTS

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July, 1948

EDITORIAL

The primary duty of an Editor, is to edit. This is defined by the Oxford dictionary as "To prepare or set in order for publication." Set out in this way his duties are light as he has merely to set in order the material sent to him, rejecting that which is unworthy and arrange that the remainder shall be published to his readers on the due date of publication. In actual fact, his problems are considerable. He sees his publication date looming close; he is bedevilled by the tardiness of the writers of his leading articles; he wonders whether that which he should reject is good enough to fill in as he is in duty bound to honour his implied promise to the advertisers of having the publication in the readers' hands on the due date, and in these days of shortages the problems of the printer press hard on him in his use of space and materials.

In no publication is the Editor in a more difficult situation than in the Editorship of a technical journal in which he may have specialised knowledge in few of the many lines of work represented in his paper so that he must take the advice and guidance of sub-editors or acknowledged leaders in the field, taking their opinion as his own and accepting the inevitability of errors large or small which may not be obvious to him. These may be errors of commission or omission, editorially or typographically, and for which he must accept all blame.

To rectify such errors and to allow his readers to express their opinions on these or other matters, he will open the pages of his publication to those who wish to write to him on matters of interest to all, hoping thereby to promote constructive criticism (which by its nature will be beyond abuse and innuendo) and so set right the original error. Only in such ways, and by the exercise of impartiality in all his actions will his Editorship be judged good.

LABORATORY PROCEDURES IN THE KAIKOURA TYPHOID EPIDEMIC

ANN L. BENNETT AND D. H. ADAMSON

(From the Pathology Department, Christchurch Hospital)

Abbreviations

S.S. agar = Shigella-Salmonella agar (Difco). Positive = Eberthella typhosa isolated or Agglutination against E. typhosa H. or O to a titre of 120 + .

TH = E. typhosa, H. antibody.

TO = E. typhosa, O antibody.

AH, AO, B = Similarly.

The typhoid epidemic which occurred at Kaikoura in 1947. and involved over 80 people, is believed to be the largest epidemic experienced amongst the white population in New Zealand. Blood for agglutination tests from the first suspected case was received at Christchurch laboratory on the evening of 9th October. This showed a TH titre of 960. Further bloods for agglutination were received on 14th (1), 16th (1), 17th (7), all of which were negative though two showed titres of TH60. On 19th, 11 bloods were received for agglutination, 5 of these were positive, all against TH, and one also against TO to the same titre. These 11 bloods and one other, all sent in unsterile containers were cultured and E. typhosa was isolated from 7 of them within 4 days. At this stage it was decided to set up a laboratory at Kaikoura Hospital, 120 miles north of Christchurch, this being done on 29th, and it is thought that an account of the bacteriological findings may be of interest.

Eighty cases are under discussion. Of these, 38 were females and 42 males. The age group incidence shows the group 0-10 yrs. to be the greatest affected one, which would seem to indicate either that immunity had not been developed or that the risk of infection was greater in this group.

In years/number affected this was 0-10/35; 10-20/12; 20-30/8; 30-40/16; 40-50/5; over 50/4.

Positive blood cultures were obtained in 42 cases and positive agglutinations in 23 cases where the blood culture was either negative or not performed. In 7 further cases diagnosis was confirmed by cultural examination of the faeces where the previous methods had failed. Of the remaining 8 cases the two earliest were diagnosed clinically, one had a titre TH30, but no bacteriological findings, and 5 were never proved in the Laboratory.

Circumstantial evidence indicated that the epidemic was a milk-borne one, but so far 6 specimens of faeces. 1 urine and 3 bloods for agglutination from only 3 persons, and 4 samples of milk and 8 water samples have been received for examination for carrier state—a'l with negative results.

Setting up Laboratory, etc.

The room used had previously been a small clinical laboratory in which the local Medical Practitioner, Dr. R. L. Withers, examined his particularly urgent specimens.

This room was $8ft \ge 6ft$ in size and was detached from the hospital by a few yards of concrete. There was a bench along one side below the window, with sink and cold water at the far end, and there were 4 wide shelves along the opposite wall. It was ar-

ranged to have the bottom shelf widened by 6 inches to form another bench. At the end opposite the door was a bench almost to the sink with incubator and 2-tube electric centrifuge. This bench was also widened, leaving a space of 5ft x 3ft between the 3 benches and door. The local Electric Power-board installed a switchboard with new leads from the mains and wall plugs for additional apparatus for a complete miniature laboratory where all the usual bacteriology and haematology was readily carried out, as well as various biochemical tests.

For the first fortnight, while there was only one of us working there, one of the main difficulties was maintaining the shuttle service in media. Owing to the lack of sterilising facilities—only a 12in. x 12in autoc'ave heated on an electric plate was available all media except carbohydrates was sent from Christchurch ready for use. There was a great shortage of containers, especially Petri dishes, and only 1-2 dozen could be exchanged each time and, of course, all cultures had to be immersed in strong lvsol and containers rinsed and packed before transmission per service car. The same difficulties were being experienced at the other end.

As it was impossible to maintain this service efficiently as the proportions of the epidemic increased and more suspects were awaiting tests, further supplies of apparatus were requested and obtained from other New Zealand laboratories, and a second Bacteriologist came up from Christchurch. The new morgue, 40 yards from the hospital, was adapted for use as an agglutination, washing up and packing room with an electric hot plate and extra lights and plugs, while the original laboratory was used for Bacteriology. blood counts, urinalysis and sterilising with a new electric autoclave. After the end of the first week, a 3ft. square Hearson electric incubator was obtained from the Christchurch laboratory to replace the 1ft. square oven type one which occasionally varied up to about 62° C. (this killed the growths on the smaller volumes of medium, but fortunately the blood cultures all survived and the original cultures of faeces in tetrathionate broth were on the bench at these times). At about the same time a cylinder of Rock Gas and an adapted Bunsen-with the jet partially blocked with solder to primus size-were obtained, and later a set was installed in the morgue.

On the clerical side as little as possible was done, compatible with complete and clear records. All results were entered in the respective books according to the nature of specimen, e.g., K/SR 47/54—Kaikoura, Serology, 1947, 54th agglutination.

Blood Cultures

Blood cultures were carried out on 57 cases suspected of typhoid fever. Twelve of these were clot cultures carried out at Christchurch Hospital before the setting up of the Laboratory at Kaikoura. The blood for these was received in clean non-sterile tubes and the clots were cultured in 1 per cent glucose broth. From these, *E. typhosa* was isolated in 7 cases. Of the other 45 cases, 35 were proved positive, and 10 negative.

The method adopted for the blood cultures at Kaikoura was as follows: The media used was Taurocholate Water (2% Sodium taurocholate + 0.5% Sodium Chloride in high grade tap water). 5-10 mls. of blood was collected from the patient in a syringe sterilised by boiling, and was placed in a blood culture bottle containing 50 mls. of the medium. The bottles used were 6 ounce media flat bottles with rubber-lined screw caps. A hole was punched in the metal cap which was covered by adhesive plaster before autoclaving with cap tightly screwed down. The blood was introduced by temporarily removing the plaster and puncturing the rubber lining with the syringe needle.

This method proved very satisfactory and very few bottles were contaminated. The culture bottle was then incubated and a sub-culture was made on to blood agar and S.S. agar plates after two days. In 10 early cases, however, E. typhosa was isolated after 24 hours' culture. Slide agglutinations with specific anti-sera (TH, TO, AH, B) were carried our with the growth from the sub-• cultures. Positive results with either TH or TO, by slide agglutination were confirmed by the biochemical reactions of the organism in sugars. Where there was no growth, after 48 hours' culture the bottles were reincubated for a further 48 hours, and then sub-cultured again; if negative after four days, the culture was discarded. From one of the clot cultures, which was incubated longer, E. typhosa was isolated after seven days' culture, but of the remaining cultures in one case only was E. typhosa isolated after three days' incubation (and this was probably due to variations in the temperature of the incubator).

Agglutination Tests

Widal agglutinations were carried out in a high percentage of the cases in conjunction with the blood cultures, chiefly because it was difficult to determine what stage of the disease many of the patients had reached, as they were nursed in their homes until hospital accommodation could be provided for them.

Widals were performed on 67 cases. A titre of 120 for E. *typhosa* H or O agglutinins was accepted as a positive result. Agglutination against the H antigen to a higher titre occurred in all but three cases. In two cases the O agglutinum titre was higher than the H titre (240,960) and in one case the O agglutinins alone were present (titre 120). In 24 cases, O agglutination was absent. Forty-one cases were positive and of these 18 had positive blood cultures the same day. Twenty-six cases had negative results, but 21 of these were proved positive by blood cultures. In 14 cases, Widals were repeated at an average of fifteen days later, and of these 9 had a rise in titre, 1 remained the same, 4 remained negative.

Two other cases which were positive on the first Widal later developed very high titres, one reaching a titre of TH 1920, and the other TH 7680. It was observed also, that in several cases where H agglutinins were present to a high titre, and the O agglutinin very low or absent, the patients became very ill, and the only two deaths in the series fell into this group. Thus: H.A. TH-1920/TO-30; G.C. TH-960/TO-0.

One case, M.W., a 23-year-old pregnant woman, had an original titre of TH 60, but later had two negative tests. Two blood cultures also proved negative. She subsequently had a positive faeces culture from the third specimen of her clearance test.

Two other cases had previously been inoculated with T.A.B. vaccine.

D B. had T.A.B. overseas in 1941 and 1942. Approximately 3 weeks after the onset of his illness he showed TH/30; TO-; AH-; AO-; B- and five days later his titre of TH agglutinins had risen to 240, the others being negative.

J.L. F.17, a nurse, had injections of T.A.B. vaccine on 2/11/47, and 9/11/47. On 21/11/47, she first became ill, and on 23/11/47 had a positive blood culture, and the Widal showed TH-; TO-; AH 480; AO-; B 480.

This case would seem to be a pointer in favour of the much debated existence of the "negative phase" shortly after inoculation with a vaccine.

The method adopted for carrying out the Widal agglutination test was as follows:----

Kahn tubes were used because of the shortage of Dreyer's tubes. The antigens used were Commonwealth suspensions of S. typhi H; S. typhi O; S. paratyphi A. H; S. paratyphi A. O.; S. paratyphi B.

Doubling dilutions of the patient's serum were made an equal volume of the suspension added to each tube and the racks placed in a water bath at 56° C for two hours ("H" suspension) and four hours ("O" suspension). To save tubes, however, the following dilutions were performed: TH and TO 1:30-1:240, AH and AO 1:30-1:120; B 1:30-1:60.

If agglutination occurred in the final tube a further series of dilutions was made and the agglutination repeated under the same conditions. One set of controls was used for each batch of 9 tests carried out simultaneously.

Cultural Examination of Faeces

Specimens of faeces were not usually examined until the patient reached the convalescent stage, and was ready for clearance. In four cases, however, positive diagnosis was established by cultural examination of faeces where other methods had failed. In three other suspected cases the diagnosis was confirmed when E. typhosa was isolated from the faeces during clearance tests. The selective media used were as follows :----

Tetrathionate broth (Difco). Shigella-Salmonella agar (Difco):—Bacto-beef extract, 5.000 gms.; Proteose- peptone, Difco, 5.000 gms.... Bacto-lactose, 10.000 gms.; Bacto-bile salts, No. 3, 8.500 gms.; Sodium citrate, 8.500 gms.; Sodium thiosulphate, 8.500 gms.; Ferric citrate, 1.000 gms.; Bacto-agar, 13.500 gms.; Bacto-brilliant green (DBg-2), 0.33 mgms.; Bacto-neutral red, 0.025 gms.; dissolved in 1000 mls. aq. dest. by boiling 1 to 2 minutes.

MacConkey's agar: Sodium taurocholate (B.D.H.), 0.5 gms.; Sugar-free peptone ("Bacto"), 2.0 gms.; Agar agar (Davis), 1.0 gm.; 1% Neutral red (aq. soln.), 1.0 ml.; Lactose (Difco), 1.0 gm.; Tap water (or aq. dest + 0.03% CaCl2), 100.0 mls.; pH 7.6 at 20°C.

The use of this medium was discontinued near the beginning as it did not seem to be very satisfactory, many lactose fermenting organisms being only slightly inhibited.

Wilson Blair agar (Difco) was used in a few cases, but we had little success with this, probably because we have had very little previous experience with it.

In most cases the faeces used for culture were fresh specimens. A piece the size of an almond was inoculated in a tube of tetrathionate broth and incubated for 18-24 hours. A sub-culture was made from this on to half an S.S. agar plate and incubated. After incubation the plates were examined carefully and suspicious colonies picked off on to small rectangles of a blood agar and S.S. agar plate. If these sub-cultures were still suspicious after incubation slide agglutinations were carried out on them. If these showed agglutination they were confirmed by the biochemical reactions of the organism in sugars. Although few cases relied on diagnosis by cultural examination of the faeces, a number of positive results were obtained during clearances of proved cases, which necessitated the clearance being stopped, and repeated a week later. Three successive specimens of faeces and urine taken at intervals of not less than 48 hours and not earlier than 4 weeks from the date of first symptoms and showing negative results, were taken as a criterion of clearance of infection (P.H.D. N. Canterbury). One of these, W.H., male, aged 80, still has positive stools after 12 attempts at clearance, and 5 months after the commencement of his disease. This man has had a previous attack of typhoid fever in the Boer War.

We encountered several difficulties in the use of the media. The tetrathionate broth did not inhibit the growth of *Proteus* and other non-lactose

fermenters. When the broth was sub-cultured on to selective media the *Proteus* appeared to be only slightly inhibited. As a result of this, tetrathionate broth was made up in Christchurch, and consisted of the following:—

1. Sterile nutrient broth, pH 7.4, 900 mls.2. Sterile chalk, 50 gms.

3. Sodium thiosulphate 50 gms, dissolved in water, made up to 100 mls. and steamed for 30 minutes.

4. Iodine 5 gms. and potassium iodide 4 gms, ground up in a mortar and dissolved in 20 mls. of distilled water.

The sterilised constituents are kept separately and mixed aseptically for use without further sterilisation.

This, however, proved little better than the Difco preparation.

Another peculiarity encountered during clearances was the incidence of an organism producing acid and gas in glucose and mannite. These results were, however, disregarded as the causative organism of the epidemic was definitely established as E. typhosa. There was neither space nor time for identification of obscure organisms which were proved by biochemical reactions and agglutinin absorption tests to be neither of the *Eberthella* nor . Salmonella group.

Cultural Examination of Urines

Urines were received in the laboratory, and were poured from their containers into tubes. These were incubated for 6 hours. They were then inoculated—one loopful from the top, and one from the bottom of the tube after decantation—on to S.S. agar plates. In one case, urine culture results during clearance have been repeatedly positive: all the others have been negative.

Additional Examinations

In addition to the work done on the 80 cases in the above series, a number of examinations were carried out on suspected cases of typhoid fever in the district.

These amounted to the following ---Blood cultures 73, Agglutination tests 64, Faeces 24.

Also 90 routine examinations including blood counts, various urine and faeces tests, throat swabs, pus, etc., were carried out.

Summary

A survey of the work carried out at the emergency laboratory set up at Kaikoura Hospital during the recent epidemic caused by *E. typhosa* and the bacteriological results obtained in 80 cases suffering from typhoid fever are submitted. The difficulties of setting up and equipping such a laboratory are discussed.

Cultural examination of the blood is described and the results showed this to be a satisfactory method for diagnosis. In 42 cases *E. typhosa* was isolated from the blood. Two previously inoculated cases are described and the value of repeating agglutination tests is shown.

Examination of faeces and urine by cultural methods are described.

Agglutination tests carried out show that, although it was of interest to test for "O" agglutinins, in this series it was of diagnostic value in only 3% of cases.

Acknowledgment

We would like to thank Dr. Pearson, Director of Pathology,

Christchurch Hospital, for his advice and encouragement and for his permission to publish this article.

CERTIFICATE OF PROFICIENCY IN BACTERIOLOGY AND CLINICAL PATHOLOGY

Medical School, Dunedin. 25-27th February, 1948. Examiners: Sir Charles Hercus, Professor E. F. D'Ath, Dr. P. P. Lynch, Dr. W. Gilmour.

PAPER

(Three hours.)

1. Describe the biological characters and the pathogenic properies of the *Clostridium tetani*.

2. How would you establish by laboratory methods a diagnosis of syphilis in the primary and tertiary stages respectively.

3. How can it be established that a person is a carrier of

(a) The meningococcus.

(b) Entamoeba histolytica.

4. (a) How would you proceed to prepare a stock of Rh positive serum for use in the Rh typing of blood?

(b) Describe the methods of performing the following tests:

(1) Bleeding time.

(2) Fragility of red cells.

(3) Haemoglobin content of blood.

(4) Occult blood in faeces.

PRACTICAL BACTERIOLOGY

(Three hours.)

1. Tubes X, Y, Z contain the cells in saline and sera of three persons, all of different blood groups. Determine to which group each belong, and describe accurately your technique and reasoning. None of them belong to group B.

2. Describe briefly the technique of the Gram stain and the Z.N. stain; upon what does the staining reactions in each technique depend.

Appropriately stain the following smears and report on your findings and state what further procedure you would carry out to confirm your diagnosis.

(a) Smear from urethral discharge. (b) Smear from a mouse inoculated from a suppurating inguinal gland in a male who has just arrived by air from India. (c) Smear from C.S.F., from a baby with meningeal symptoms. (d) Smear from a culture from an infected wound. (e) Smear from a culture of a non-lactose fermenting organism from a water supply. (f) Smear from an ulcerating throat. (g) Smear from an exudate from a dead sheep. (h) Smear from a C.S.F. from a meningitis case. (i) Smear from a mouse inoculated with material from a case of pneumonia.

(j) Smear from pus from a suppurating gland in the neck.

(The examinees state these to have suggested (a) Gonocoscus. (b) Plague. (c) Pneumococcus. (d) Anaerobic bacillus. (e) gram negative bacillus. (f) Vincent's organisms. (g) Anthrax. (h) Meningococcus. (i) Friedlander's bacillus. (j) T.B.).

3. Write notes on items K-S (15 minutes for all). (The examinees suggest that these were: (k) Ascaris lumbricoides worms. (1) Sarcoptis scabei female. (m) Echinococcus granulosa worms. (n) Leishman Donovan bodies. (o) Darkground condenser. (p) Ova of A. lumbricoides and T. trichiura. (q) Anaerobic chamber. (r) Ringworm spores. (s) A filar micrometer eyepiece.

PRACTICAL PATHOLOGY

(Three hours)

- 1. How would you proceed to prepare a stock of this preparation for use in Hospital wards?
- 2. What are pyrogens? How would you ensure that this preparation was as pyrogen free as possible?
- 3. What is this piece of apparatus? Suggest possible uses for it in a Hospital Laboratory.
- 4. How would you sterilise these substances?
- 5. What are the principles which underlie the use of this instrument? What routine care would you give the instrument in order to maintain it in good working order?
- 6. Briefly outline the mode of preparation of these reagents.
- 7. Stain the film provided by Leishman's stain and do a differential count.

(The examinees state these to have been (1) Isotonic 0.9 per cent saline. (2) 5 per cent glucose saline. (3) Colorimeter. (4) Creta gal. pulv., rubber gloves, $\frac{1}{2}$ per cent procaine, sulphanilamide powder. (5) Colorimeter. (6) White cell diluting fluid, Benedict's qualitative solution, 2/3N sulphuric acid).

ORALS

The following questions were among those asked by the examiners:----

Sir Charles Hercus: The use of the guinea pig in laboratory work. The cultivation of Br. abortus.

Professor D'Ath: Blood calcium, uric acid, phosphates, micro blood sugar, accuracy of copper sulphate, specific gravity method for proteins, colorimeters, Beer's law, standardisation of solutions, normal solutions, spectrophotometers, fimits of accuracy of colorimeter, pH and buffer solutions, accuracy of pH meters, pH of distilled water, anticoagulants for blood, routine methods of staining sections, mounting museum specimens, sharpening microtome knives, penicillin, stability and diluents.

Dr. Lynch: T.N.P.N. serum proteins, protein precipitants laboratory animals and their uses, serum for Loeffler, trichinosis,

postmortem picture of guinea pig infected with *Br. abortus*, blood calcium, total protein in C.S.F., *Haemophilus* group, findings in C.S.F. in poliomyelitis, *Cl. tetani* and findings in guinea pig, cough plates for pertussis.

Dr. Gilmour: The blood bank, length of survival of spirochaetes in banked blood, Weil's disease, its diagnosis, Hookworm, urine analysis, bacilluria, diagnosis of typhoid, O.H. and Vi antigens, group A streptococci, penicillin sensitivities.

COUNCIL MEETING

This was held on May 1st, 1948, at Wellington, all members of Council being present.

The following were admitted as members:—Senior: Mr. H. G. Bloore (Blenheim), and Junior: Misses Joan M. Winter and Patricia J. Bellingham (Auckland), and Messrs. H. J. Little. Frederick M. Rush-Munro, E. Robinson, R. B. Stockwell and J. Spencer Cole (Auckland); Walter Todd (Palmerston North); and Messrs. F. L. N. Corey and R. L. Bennett (Christchurch).

Resignations were received with regret from Mr. W. N. Nuttall (Wellington), M. G. Jenner (Dunedin); and Misses Helen Murchie (Wanganui) and D. Smaill (Auckland).

A letter was received from the Institute of Medical Laboratory Technology, England, and the Secretary was instructed to write thanking them for their fraternal greetings.

The registered office of the Association was changed to 139 Kohimarama Road, Auckland, E.1., as the Journal is now printed and published there.

A typewriter was purchased for the use of the Secretary-Treasurer.

The remainder of the meeting was taken up by routine matters and arrangements for the forthcoming conference as well as instructions to special Committees.

NOTES FROM THE PRESIDENT

Members will have been wondering what the Council of the Association has been doing since last Conference; here is a brief summary,

A Council meeting was held in mid-November at which the whole question of salaries and conditions of cmployment was gone into in pre-paration for the meeting of Messrs. D. H. Adamson, D. Whillans and myself as representatives of the New Zealand Association of Bacteriologists with the other members of the Bacteriological Officers' Salary Advisory Committee. This meeting was held in the early part of December under Dr. M. H. Watt as Chairman, with Messrs. W. E. Bate and J. W. Dove as Hospital Board members, and Mr. A. V. Keisenberg as Officer of the Public Service, and a large measure of agreement was reached on all questions. Details of the new scale and conditions of employment are regarded as confidential, but are in line with those passed by our last Conference. Delay has occurred in the gazetting of the new scale because of difficulties in the framing of rules and regulations regarding the grading of senior bacteriologists, but a further meeting of the Advisory Committee held in Wellington on June 15th, is expected to resolve all difficulties. Although the discussions seem protracted, we are fortunate in being one of the first groups of employees under the Hospital Boards to be considered. A report on the above discussions was given to the Council of the New Zealand Association of Bacteriologists at a Council Meeting held in Wellington on May 1st.

Mr. D. Whillans and I represented the Association at the Pathologists' Conference in Wellington on Wednesday afternoon, May 26th. We presented the Association's views on the scope of the Senior Syllabus, the proposed Intermediate Syllabus, the Title of the Senior examination for Hospital Bacteriologists, and Refresher Courses for Hospital Bacteriologists. We were received in a very cordial manner, and after stating our case were asked questions on various matters with which we had dealt. Our last request was for a representative Committee of Fathologists and Bacteriologists to discuss these matters. This was held in the afternoon of June 15th, and was an official meeting held by the direction of the Department of Health. Drs. Mercer and Pullar represented the pathologists, and Mr. Whillans and I the bacteriologists. Here again good progress was made, and it may be possible to give a further report to the Conference on this matter.

Only a few days ago all Hospital Boards which have laboratories, received a circular from the Health Department asking that all members of laboratory staffs be given a medical examination and X-ray examination before taking up duty and an annual chest X-ray thereafter.

We understand that the £25 cost of living increase has been granted to all those members who have applied for it, the increase dating from October 1st, 1947.

CONFERENCE, 1948.—Every laboratory should be fully represented at Auckland. The Conference Committee has arrangements well in hand, and you are assured of a profitable and interesting time in the Queen City. We hope that all who cannot be present will take full advantage of the proxy voting so that an Executive Council that has the support of the majority of our members will be elected for the ensuing year.

E. L. F. BUXTON.

HERE AND THERE

Miss P. Bellingham, B.H., Sc., Miss J. Winter and Mr. H. J. Little joined the staff in January of this year. Mr. Little has had some ten years' previous experience on the clerical side of Hospital Administration.

Mrs. H. Oliver, who joined our staff in October, 1943, will be leaving us at the end of this July and will carry with her our best wishes for the future. Her willing help will be missed.

The Auckland Hospital Board's Laboratories "Food for Britain" campaign mentioned previously in an earlier 'ssue of th's Journal has been responsible for sending food parcels to all members of the Laboratory staffs of the Radcliffe Infirmary, Oxford. St. Bartholomew's Hospital, and St. Thomas's Hospital, London. The many letters received thanking us for the parcels have more than repaid us for our efforts.

The "Auckland People" are busy with the preparations for the coming Conference of the New Zealand Association of Bacteriologists. The preparations are progressing in fine style, and we are looking forward to meeting and entertaining our prospective visitors in July. P.B.S.

Dr. C. T. B. Pearson has resigned to take up a position with Dr. P. P. Lynch, of Wellington, and Dr. G. C. T. Burns and N. Godfrey have been appointed Assistant Pathologists. Dr. Burns has just returned from a course in England after some years in the army.

Mr. R. Bridger, the Hon. Secretary of the Junior Sub-Committee, has joined C.O.R.S.O. as a Bacteriologist. D.H.A.

LETTERS TO THE EDITOR

Dear Sir,

May I be permitted to criticise an article on "An Atypical Member of the Salmonella' Group," which appeared in the April issue of the Journal. In the article assumptions have been made with an incomplete factual basis. Firstly, the organism has been termed a member of the Salmonella group on slender evidence, based partly on biochemical behaviour, but hinging on serological relationship to the group. Certain requirements must be fulfilled by each organism before it can be included, as a member. Although the classification of Salmonellas cannot be based solely on antigenic analysis nor even less on biochemical behaviour, both may be fitted into what may be termed the jig-saw of the Salmonella pattern.

BIOCHEMICAL EVIDENCE.—Biochemically there are other gram negative bacilli within or even unrelated to the group which may show these reactions. Assuming that the organism in question was *S. typhi-murium*, the variation in biochemical behaviour is quite common. Of 40 cultures isolated in this laboratory during 1945, only 27 were found to be biochemically typical.

ASSUMED SEROLOGICAL EVIDENCE .- Before an organism can be classified as a Salmonella, both the somatic and flagellar antigens must be shown to be present. In the article, the somatic antigens only of group (B) were demonstrated. The flagellar agglutination with polyvalent Salmonella (H. specific and non-specific) is of little value, as some of these antigens are found in unrelated organisms. In addition the somatic antigens of group (B) I, IV, V and XII have been found singly and in groups in the pseudosalmonellas, groups "1"-"5." Therefore, it can be stated that the serological evidence for the existence of a salmonella type is not complete and furthermore that these somatic factors may occur in unrelated types. Secondly, the organism has been presumed to be S. typhi-murium. The biochemical behaviour is of small value, as it could fit many other types. There was still less serological evidence for this assumption, as the important specific and non-specific flagellar factors were not demonstrated. Therefore the author's assumption cannot be accepted. As far as practical purposes are concerned, all that is necessary for the provisional diagnosis of S. typhi-murium, is that the specific flagellar factor (i), the non-specific factors (1, 2...) and the somatic antigens characteristic of group (B), are present. Thirdly, although one may concede the author was unfortunate in not having a range of specific sera, one may not condone the omission to include an agglutination with S. schottmuelleri (H) (S. paratyphi B) serum, specific or non-specific or even mixed, as this organism may quite well be S. schottmuelleri, on the biochemical and serological evidence so far presented. This would have shown in the (H) mixed serum a partial or complete agglutination dependent on the type of organism and on the phase present. The (H) non-specific serum factors 1, 2 ... would show complete agglutination, while the presence or absence of the specific factor "B"

dependent again on the phase, would confirm or exclude this organism. Presumably in the article, S. paratyphi (Bergey) refers to S. paratyphi A, and is not a misprint for S. paratyphi B If so, the author's statement "The agglutination of both S. paratyphi B (O) and S. aertrycke (O) antisera was explained by the fact that the two organisms have common (O) antigens, while the inability to agglutinate S. paratyphi (H) antiserum indicated that the organism was most probably S. aertrycke," is an hypothetical one only, without any foundation. There has been neither mention of a motility examination nor any attempt at suppressive phase culturing made in the article. Agglutination for the somatic factors found in group B, but common to groups (A), (D), (E3), (F3), (F4) of the Kauffman schema has not been carried out. One is also at a loss to explain the negative somatic agglutination for S. typhi (O) in the second trial of the organism. Does this mean a loss of factor XII, an antigen widely distributed in types and common to groups (A), (B) and (D) of the schema? The nomenclature used in the above is that of the International Nomenclature Committee for Microbiology. Synonyms with reference to the article and criticism :---

S.schottmuelleri: S. paratyphi B. S.typhi-murium; S.aertrycke. S. paratyphi: S.paratyphi A. S.typhi: S.typhi.

There are several other important points in the article one may wish to discuss, but space does not permit it.

Yours faithfully,

Pathology Dept., Public Hospital, Palmerston North, 16/5/48. S. O. JARRATT,

Dear Sir,

With your permission, I would like to pass criticism upon two of the articles appearing in the last issue of your Journal (Vol. 3, No. 2).

To date, in my opinion, the Journal has been of a very high standard in spite of the difficulties you have had to overcome, and this also applies to the remainder of the last issue. There have been details which, at times have left something to be desired, but the set-up, in particular, has always been and still is excellent.

The article entitled "An Atypical Member of the Salmonella Group," by J. Callaghan, is ambiguous and inadequately describes the organism. It is not stated whether 24 hours was the maximum time allowed for incubation of carbohydrate media, or whether only four varieties of these media were used.

Agglutination of the organism by Salmonella paratyphi B (O) and S. aertrycke (O) antisera mercly indicated that the organism belonged probably to Salmonella Sub-Group B, or was possibly Bacterium coli 3, a Flexner or a Pasteurella organism, though agglutination against polyvalent Salmonella (H) antiserum confirmed its inclusion in Salmonellas as suggested. The suggestion, without supporting evidence, that the organism was "most probably S.aertrycke", leaves the reader unconvinced of the reliability even of the reactions given. This is a pity, as the author has been at some pains to attempt to classify the organism as far as possible without individual specific Salmonella antisera, and it is to be hoped that we will hear more of his work.

The article entitled "Some Practical Notes of Interest," by D. F. Creed, is carelessly constructed and two of the five sections in it are of little practical interest in a general hospital laboratory. It would have been more polite to the authors concerned, had Mr. Creed given a list of references in "Recent Advances in Clinical Pathology," 1947—in which the five sections appear—saying that. in his laboratory, some of the methods had seemed satisfactory. The original text is much more comprehensible than are Mr. Creed's modified transcriptions.

Trusting you will receive more numerous articles of the standard of those appearing in your previous Journals,

I am,

Yours faithfully,

Pathology Dept., Public Hospital, Christchurch, C.1., 16/5/43. D. H. ADAMSON.

Dear Sir,

I have read with some measure of surprise the astonishing acrobatics of an organism of the *Salimonella* group which was described by Mr. J. Callaghan and published in the last issue of your Journal.

I would compliment the author on his excellent choice of substrata, in this laboratory we prefer to use those recommended by Professors W. S. Wilson and Kauffmann.

Was there some dark reason, other than that the worker would deceive himself, that he did not examine the organism for motility, indole production, utilisation of citrate as a source of carbon, H2S production; were his Durham tubes intact; was he not intrigued by behaviour of the organism on examining his substrata to be assured that they actually contained glucose, lactose, sucrose and mannitol?

I assure the author that there is nothing in his serological findings that would suggest more than that the factors (1)? IV, (V)? and XII were present; furthermore, no information of the source, the titre or dilution of the serum at which agglutination occurred were given.

One should extend sympathy to the author because his perplexity must have been akin to that of Massini when he described *B.coli mutabile*.

May I also point out that on pp. 31 and 32 of the same Journal, Mr. D. F. Creed refers to cultures of N.gonorrhoeae with a scientific approach

which, to say the least, has "gonostray."

Yours faithfully,

Pathology Dept., Public Hospital, Wellington, 4/6/48. H. T. G. OLIVE.

J. J. G. PEDDIE.

Dear Sir,

We have used the agglutination test for *Br. abortus* on whey for many years in this laboratory, and usually carry it out in conjunction with the culture methods elaborated in an article by myself in the first issue of the Journal.

We allow the milk to stand 2-3 hours to allow the cream layer to separate and then withdraw 10-20 ccs. of the milk from under this layer into a tube to which has been added 5-6 drops of rennet. After incubating for one hour at 37 degrees C (or 45 degrees C for the same time), good separation of the whey will have taken place. If sufficiently clear, the whey is used for dilutions which double from 1/25 to 1/200. If the whey is not clear it is centrifuged and the supernatant is used for testing. The *Br. abortus* emulsion is added and the rack incubated at 37 degrees C for 48 hours before final reading. Complete agglutination in 1/100 is looked upon as highly suspicious and 1/200 as definitely positive

As Br. abortus is excreted only intermittently, more reliance can be placed on the whey agglutination test where the reaction persists at the same titre from day to day.

Yours faithfully,

Animal Research Station,

Wallaceville, P.B., Wellington.

Dear Sir,

I regret that in my note on a member of the Salmonella group in the last issue of the Journal, I expressed myself in such a manner that I have been misinterpreted as giving an incomplete discourse on the Salmonella group as a whole. Unfortunately, S.paratyphi was also a misprint for S. paratyphi B.

It would seem from the letters received on the subject that the organism has not even been accepted as a member of the Salmonella group in spite of the sugar reactions (which were, in fact, as stated) in combination with the possession of both H and O Salmonella antigens. I think I would be in eminent company in asking what further tests would definitely exclude this organism from the Salmonella group. To save further space in this paper, I would not accept the apparently knowledgeable but really meaningless reply giving a combination of antigenic structures and a full range of biochemical tests as it is my belief that in dealing with the Salmonellas many persons delude themselves that a large array of agglutinations, absorptions and biochemical reactions are the ultimate in scientific attainment. Except in the fields of pure academic research or of worth while epidemiological studies, both of which require a special institution and staff, I fail to see the present worth of all but the simpler procedures. Yours faithfully,

Department of Pathology, Auckland Hospital, 18/6/48. J. CALLAGHAN.

Dear Sir.

I would point out to readers of the Journal that it is easier to criticise those who contribute to the Journal than those who do not and would further add that although my references are to be found elsewhere, it was thought of interest to report on their practical value.

21/6/48.

Yours faithfully,

D. F. CREED.

From the Mater Misericordiae Hospital,

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